FIG. 1

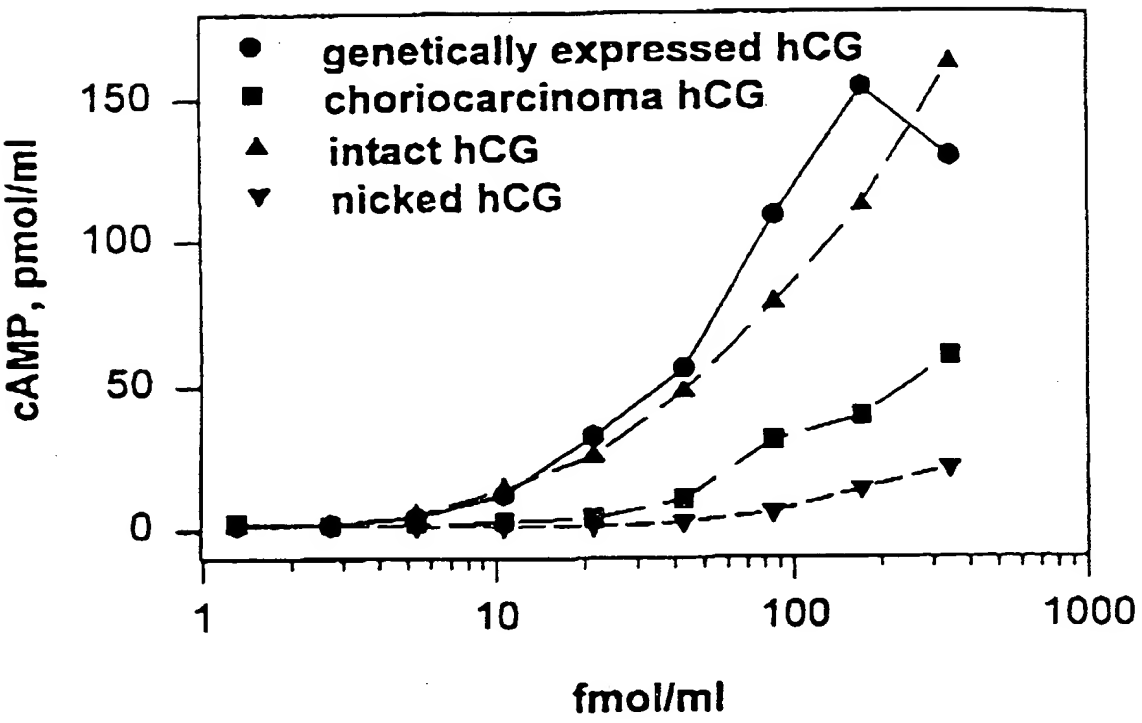

genetically expressed hCG
choriocarcinoma hCG
intact hCG
nicked hCG
cAMP, pmol/ml
150
100
50
0
1
10
100
1000
fmol/ml

FIG. 2A

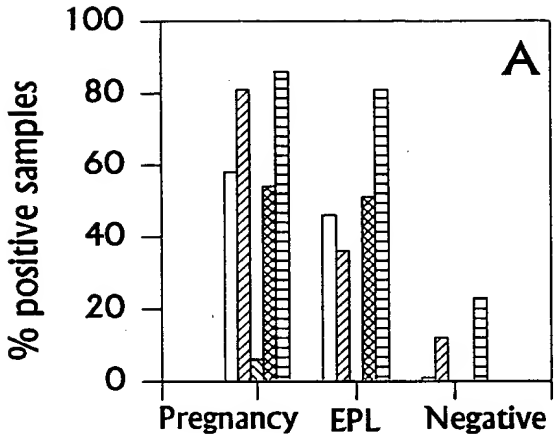
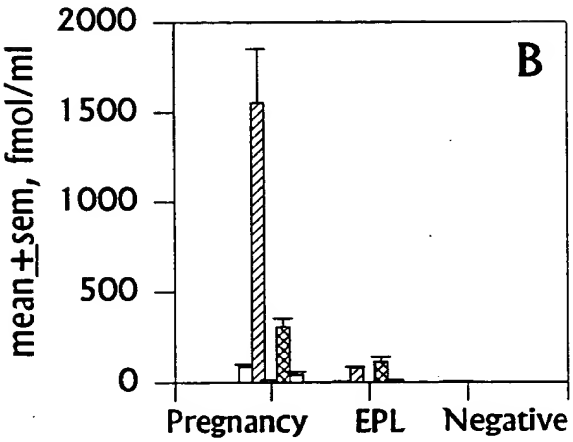


FIG. 2B



hCG (B109-B108*)
hCG (B152-B207*)
nhCG (B151-B207*)
hCGβ (B201-C104*)
hCGβcf (B210-B108*)

FIG. 3A

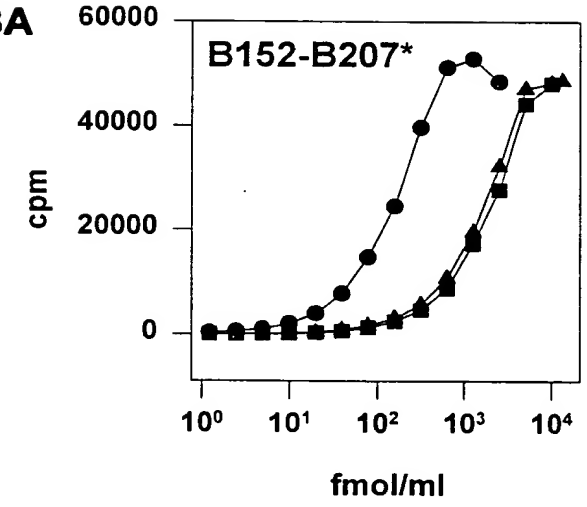
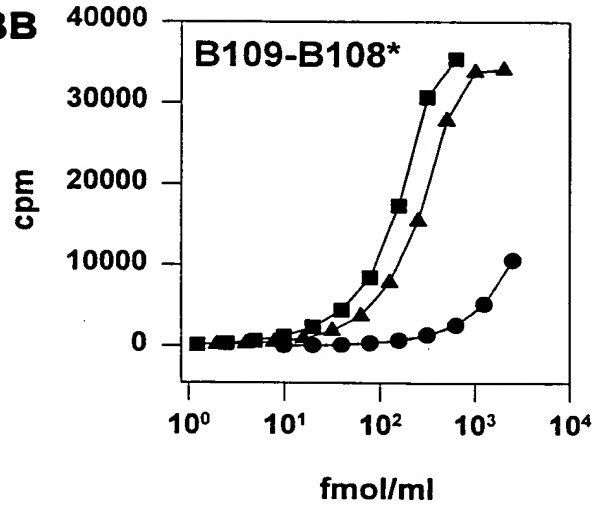


FIG. 3B



choriocarcinoma hCG (C5)

pregnancy hCG

pituitary hCG

FIG. 4

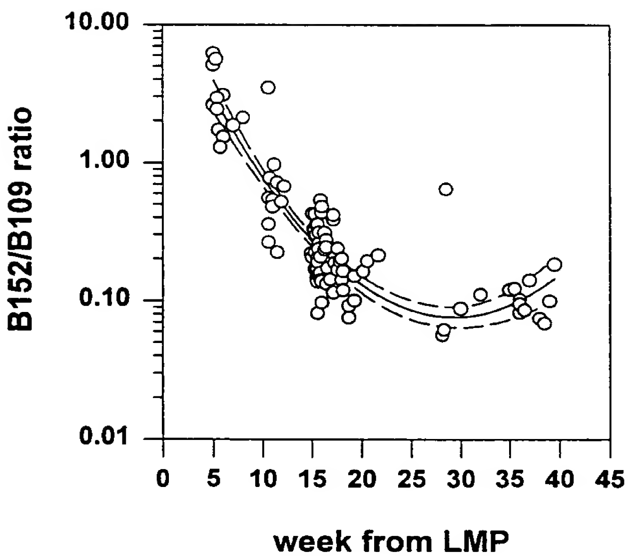

10.00
1.00
0.10
0.01
B152/B109 ratio
0
5
10
15
20
25
30
35
40
45
week from LMP

FIG. 5

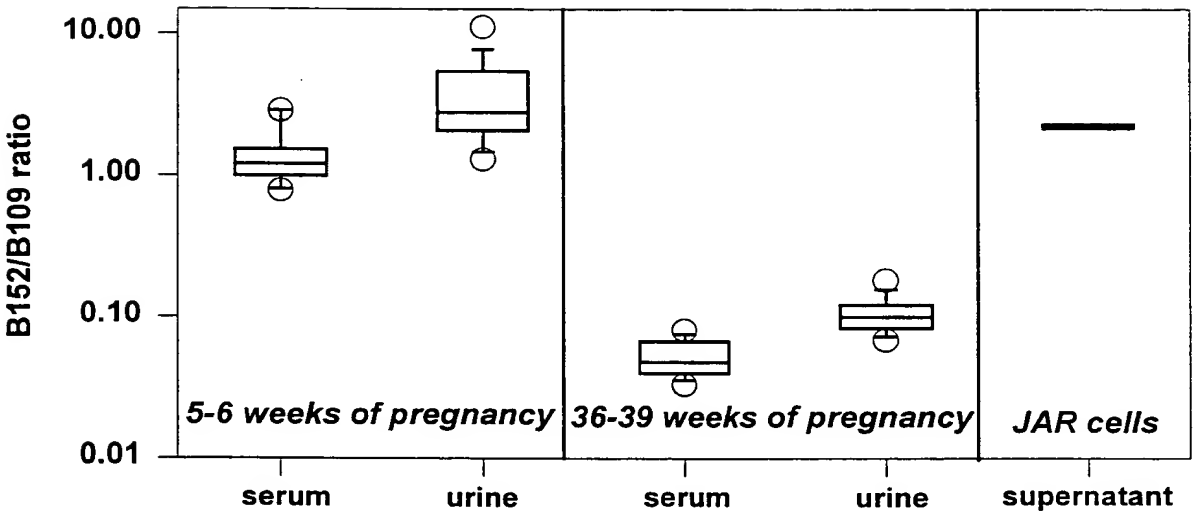

B152/B109 ratio
10.00
1.00
0.10
0.01
5-6 weeks of pregnancy
36-39 weeks of pregnancy
JAR cells
serum
urine
serum
urine
supernatant

FIG. 6

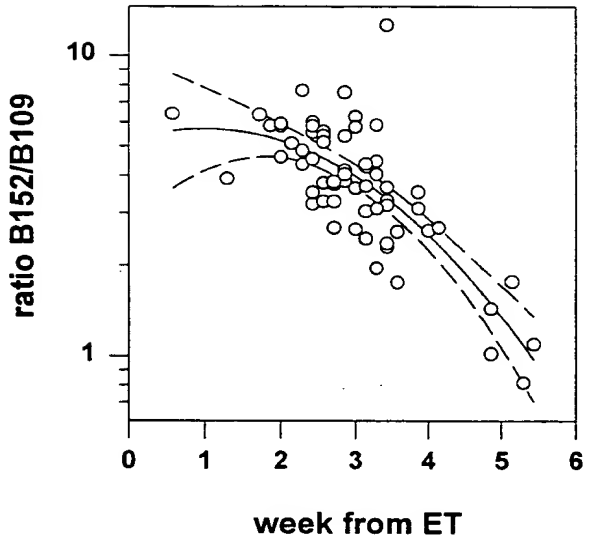


FIG. 7A

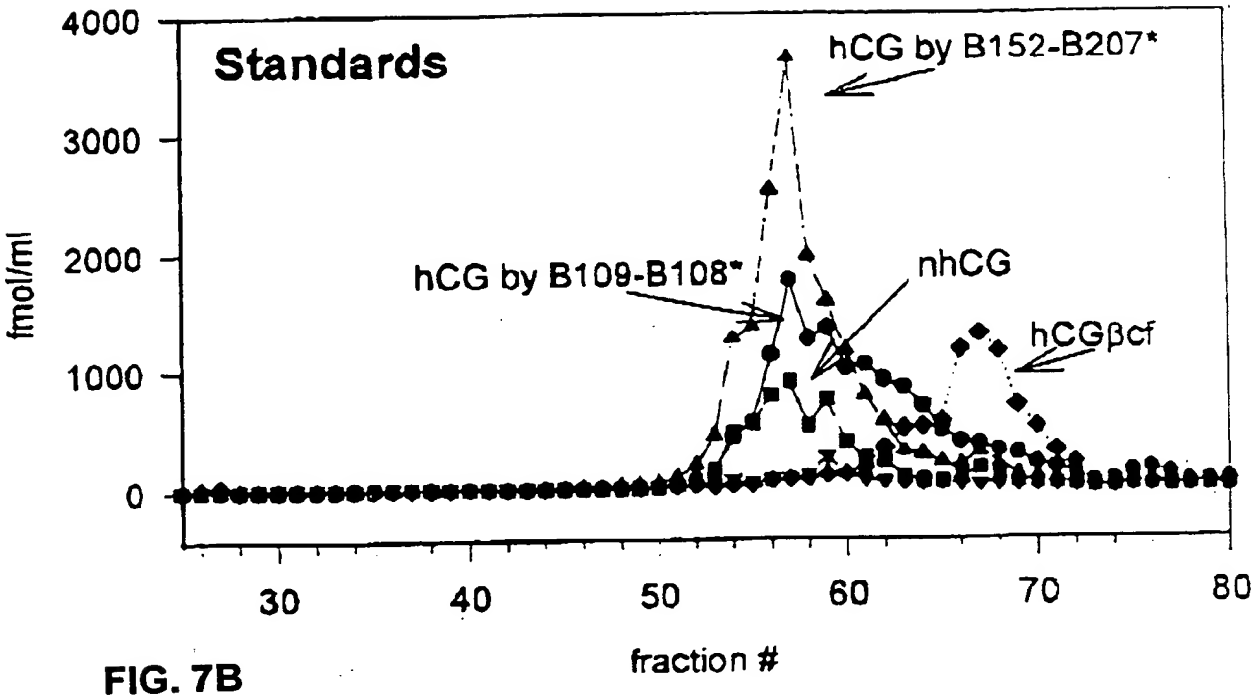

Standards
hCG by B152-B207*
hCG by B109-B108*
nhCG
hCGβcf
fmol/ml
4000
3000
2000
1000
0
30
40
50
60
70
80
fraction #

FIG. 7B

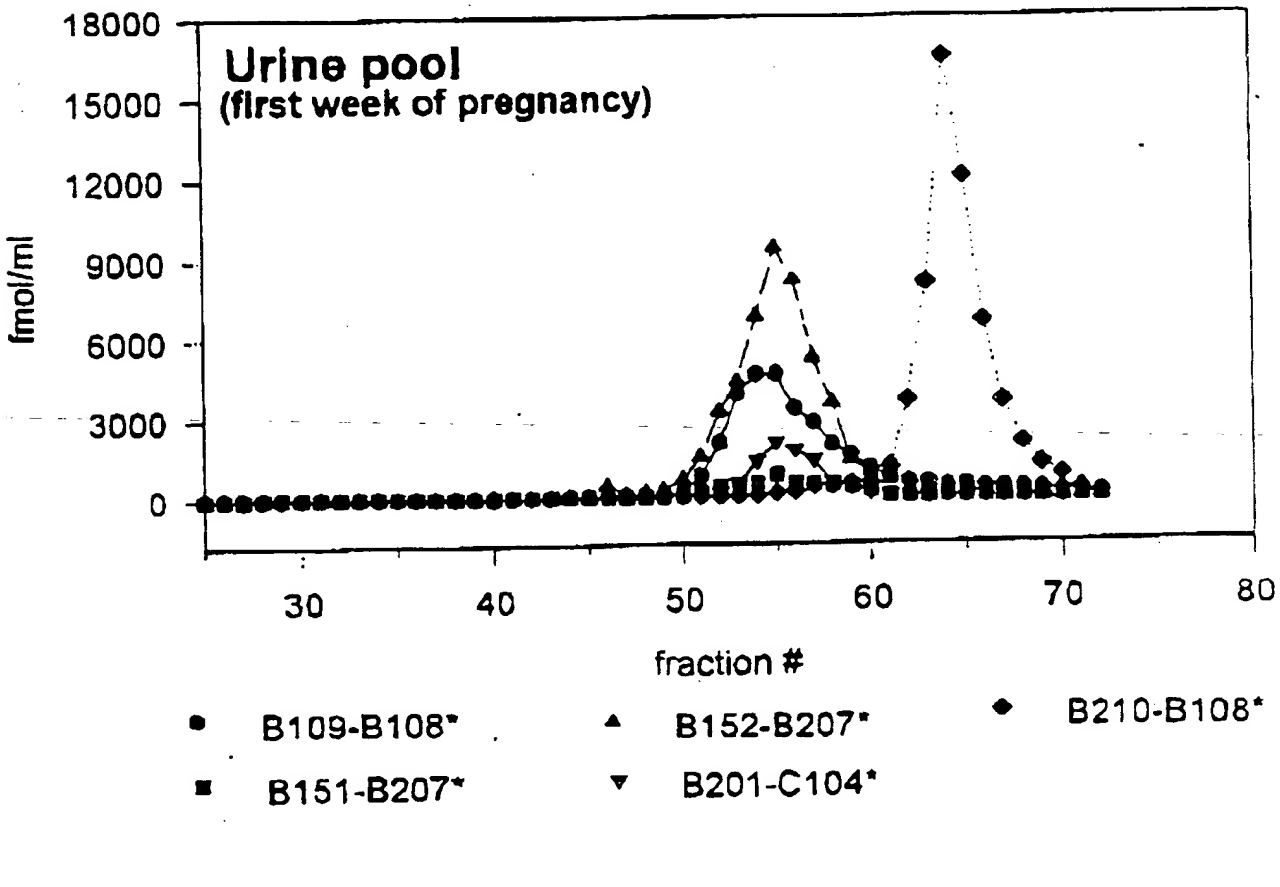

Urine pool
(first week of pregnancy)
fmol/ml
18000
15000
12000
9000
6000
3000
0
30
40
50
60
70
80
fraction #
B109-B108*
B152-B207*
B210-B108*
B151-B207*
B201-C104*

FIG. 8A

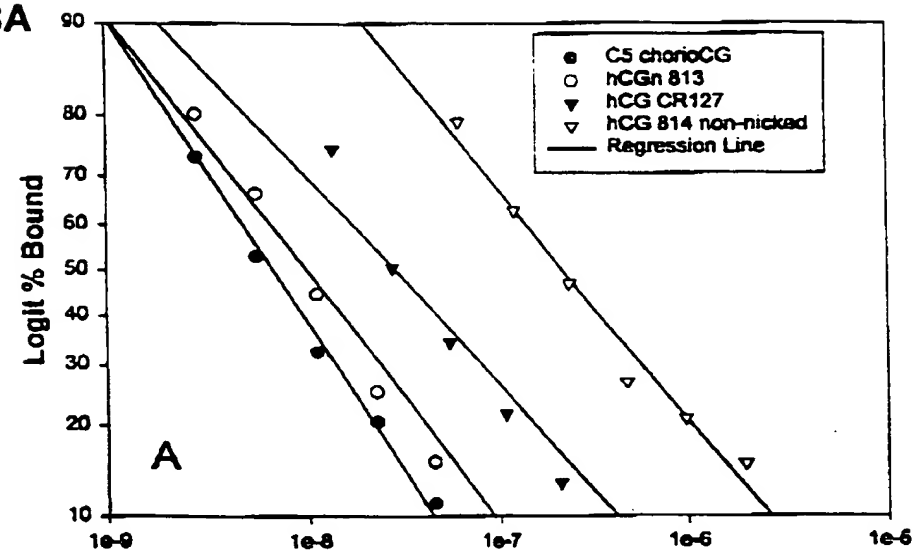

C5 chorioCG
hCGn 813
hCG CR127
hCG 814 non-nicked
Regression Line
Logit % Bound
90
80
70
60
50
40
30
20
10
A
1e-9
1e-8
1e-7
1e-6
1e-5

FIG. 8B

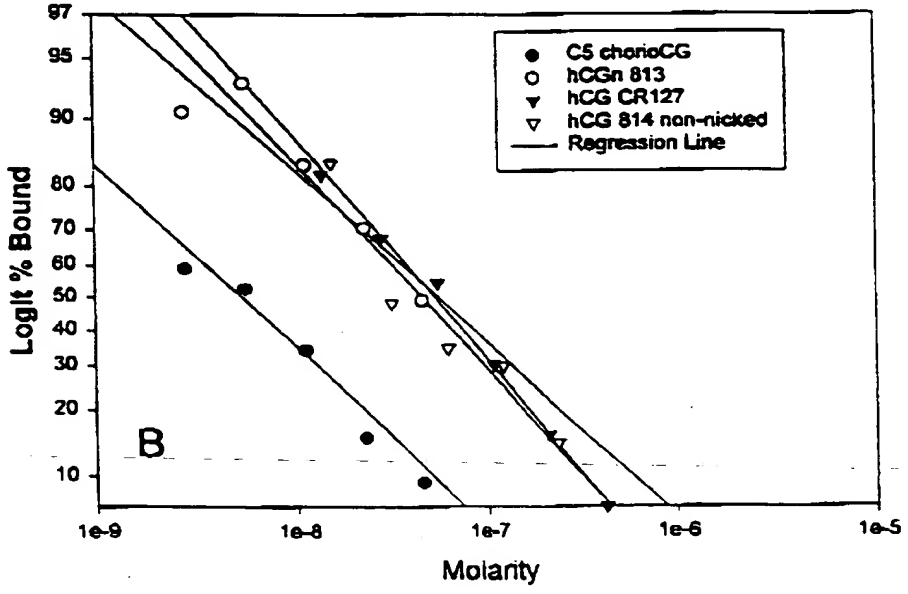

C5 chorioCG
hCGn 813
hCG CR127
hCG 814 non-nicked
Regression Line
Logit % Bound
97
95
90
80
70
60
50
40
30
20
10
B
1e-9
1e-8
1e-7
1e-6
1e-5
Molarity

FIG. 9A

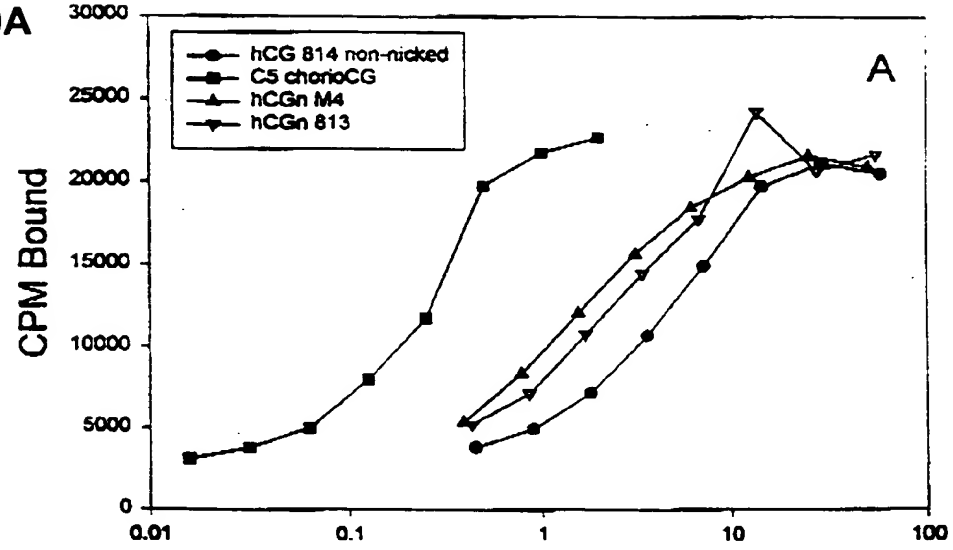

hCG 814 non-nicked
C5 chorioCG
hCGn M4
hCGn 813
A
CPM Bound
30000
25000
20000
15000
10000
5000
0
0.01
0.1
1
10
100

FIG. 9B

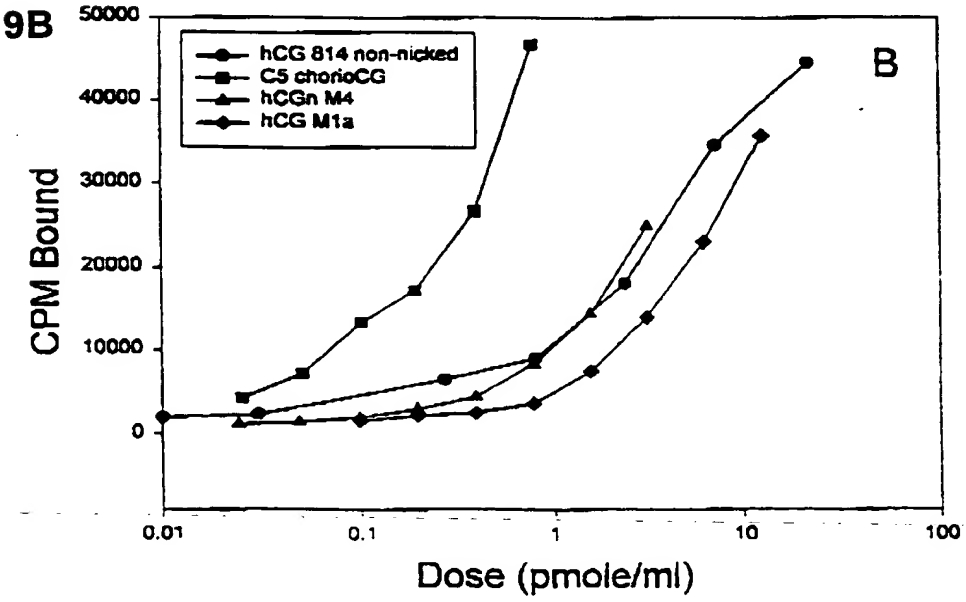

hCG 814 non-nicked
C5 chorioCG
hCGn M4
hCG M1a
B
CPM Bound
50000
40000
30000
20000
10000
0
0.01
0.1
1
10
100
Dose (pmole/ml)

FIG. 10

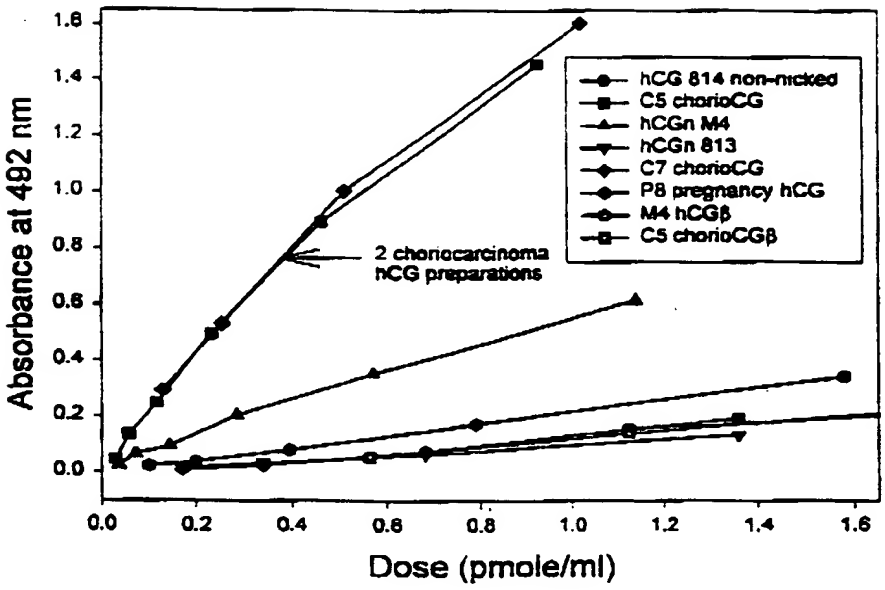

Absorbance at 492 nm
Dose (pmole/ml)
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
1.6
hCG 814 non-nicked
C5 chorioCG
hCGn M4
hCGn 813
C7 chorioCG
P8 pregnancy hCG
M4 hCGβ
C5 chorioCGβ
2 choriocarcinoma hCG preparations

FIG. 11 Table I. Characteristics of the Reagents Used to Define Antibody Specificity. The peptide and carbohydrate structures of the reagents used were determined earlier (26). The % nicked β-subunit refers to the proportion of molecules with cleavages (missing peptide bonds) in the region β43 to β48. The % tetrasaccharide core is the proportion of O-linked oligosaccharides with tetrasaccharide (vs. disaccharide) core structure, and the % sialic acid, is the proportion of O-linked structures with antennae terminated by sialic acid residues. The proportion of triantennary N-linked oligosaccharides on β-subunit is given, as is the corresponding % sialic acid.

Name	Source	N-Sialic acid [a]	O-Sialic acid[b]	% triantennary N-linked on β	% tetrasaccharide O-linked core	% β nicking
814 hCG	CR 127 hCG[b]	95	66	5	19	19
C5 chorioCG	Choriocarcinoma	95	58	48	100	100
M4 mole CG	Mole pregnancy	120	49	30	20	98
813 hCGn	CR 127 hCG	ND	ND	ND	ND[d]	80
C7 chorioCG	Choriocarcinoma	68	53	48	69	3
P8 hCG	Pregnancy	94	73	21	13	0
M4 mole β	Mole pregnancy	120	49	30	20	98
C5 chorio β	C5 chorioCG	95	58	48	100	100
CR 129 β	CR 129 hCG	96	63	11	17	19
HLH I-1	Pituitary hLH	ND	NA[d]	NA	NA	NA
M1A	Mole pregnancy	98.5	NA	16.5	<15% CTP[e]	24

[a].% sialic acid residues per sugar chain, N-linked on β.

[b]. % sialic acid residues per sugar chain, O-linked on β.

[c] The "CR" series of hCG reference preparations were made at Columbia University and were distributed internationally as reference materials for purified hCG. CR 119 is also known as the 3rd international immunoassay reference preparation for hCG.

[d]. ND is not done; NA is not applicable to that reagent.

[e]. Less than 15% of the beta COOH-terminal region is present on this preparation.

FIG. 12

Table II: Affinity Constants[a] Determined by Liquid Phase Competition Assays Using C5 as Tracer Ligand

	Competitors			
Antibody	C5 chorioCG	Nicked hCG CR 127 (813)	Parent CR 127 hCG[b]	Nick-free hCG C R127 (814)
B151	4.4×10^8	3.8×10^8	4.2×10^7	1.3×10^7
B152	3.5×10^8	5.4×10^7	4.7×10^7	5×10^7

[a]Ka as L/M

[b] hCG CR 127 is an NIH-distributed hCG reference preparation produced at Columbia University.

FIG. 13A

Table III: Matrices of data for binding characteristics of different pairs of detection antibodies using B151 or B152 as capture antibody.

A. Relative Cross-Reactivities of Two Site Assay Using B151 as Capture Antibody

Ligand	B207[a,c]	B204[a]	B201[a]	B108[a]	B109[a]	A109[a]	CTP104
C5	100%	<[b]	<	100%	<	<	100
813 CR 127 hCGn	100%	<	<	100%	<	<	47%
814 CR127 hCG	12%	<	<	37%	<	<	14%
HCG β	2%	<	<	2%	<	<	<
C5 β	5%	<	<		<	<	<
HCGβ core	<	<	<		<	<	<
HLH	2%	<	<	3%	<	<	<
HLHβ	5%	<	<		<	<	<
HCG α	<	<	<	3%	<	<	<
Maximum binding[b]	50%	0%	0%	13%	0%	0%	83%

[a] labeled detection antibodies

[b] < out of low range detection

[c] this particular assay format was applied in O'Connor et al (25).

FIG. 13B

B. Relative Cross-Reactivities of Two Site Assay Using B152 as Capture Antibody

Ligand	B207[a,d]	B204[a]	B201[a]	B108[a]	B109[a]	A109[a]	CTP104[a]
C5	100%	100%	94%	42%	53%	100%	<
813 CR 127 hCGn	10%	<[b]	<	15%	32%	64%	<
814 CR127 hCG	7%	<	<	30%	100%	26%	<
HCG β	6%	20%	19%	11%	<	<	<
C5 β	190%	100%	100%	100%	<	<	<
HCGβ core	<1%	<	<	<	<	<	<
HLH	<1%	<	<	<	<	<	<
hLHβ	<1%	<	<	<	<	<	<
HCG α	<1%	<	<	<	<	<	<
Maximum binding[b]	64%	2%	44%	80%	2%	14%	25%

The molar quantity of ligand required to produce binding equal to 50% of the maximum binding achieved by C5 was determined. Cross-reactivity shown in this table as a percentage is calculated by dividing the molar quantity of the standard by the molar quantity of the other ligand at 50% maximum binding dose.

[a] labeled detection antibodies

[b] maximum binding represents the total quantity of radiolabeled detection antibody which can bind to the plate in the system described.

[c] < out of low range detection

[d] this particular assay format was applied in O'Connor et al (25).

FIG. 14

Table IV. Immunoreactivity of antigens in the B152 immunoradiometric assay. The dose-response curves used to provide data for this table are shown in Figure 3. Each curve was fitted with 4-5 points. Slope and coefficient of determination (R^2) were determined using a non-linear regression algorithm. Slopes were used as an indicator of antigen potency. Relative potency was estimated as the slope of antigens relative to the slope of C5 Choriocarcarcinoma hCG (the immunogen).

Reagent	Slope [a]	S.E.	R^2	Relative Potency
814 hCG	0.1588	0.0098	0.992	10.2%
C5 chorioCG	1.5603	0.1015	0.983	100%
M4 mole hCGn	0.5317	0.0240	0.992	34%
813 hCGn	0.0986	0.0021	0.999	6.3%
C7 chorioCG	1.4515	0.1246	0.985	93.0%
P8 hCG	0.2192	0.0031	0.999	14.0%
M4 mole hCGβ	0.1038	0.0069	0.991	6.67%
C5 chorioCGβ	0.1286	0.0042	0.998	8.24%
CR 129 hCGβ	Only 2 points			
HLH batch I-1	No response			

[a] Slope are from figure 3 as calculated in Sigmaplot 4.01 by linear regression analysis. Units of slope are pmole/ml absorbance at 492nm.